FORM PTO-1390 (REV. 12-2001) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY 'S DOCKET NUMBER: O-1999.475 US

U S APPLICATION NO. (If known, see 37 CFR 1 5): 10/049393 To be assigned

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP00/07694	7 Aug 2000	13 Aug 1999

TITLE OF INVENTION
USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG INDUCED NEUROMUSCULAR BLOCK

APPLICANT(S) FOR DO/EO/US
Antonius BOM et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [X] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [ ] This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. [X] The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. [X] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. [ ] is attached hereto (required only if not communicated by the International Bureau).
   b. [X] has been communicated by the International Bureau.
   c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US).
6. [ ] An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. [ ] is attached hereto.
   b. [ ] has been previously submitted under 35 U.S.C. 154(d)(4).
7. [X] Amendments to the claims of the International Aplication under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. [ ] are attached hereto (required only if not communicated by the International Bureau).
   b. [ ] have been communicated by the International Bureau.
   c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   d. [X] have not been made and will not be made.
8. [ ] An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)).
9. [ ] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] An English lanugage translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 to 20 below concern document(s) or information included:

11. [X] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [ ] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [X] A FIRST preliminary amendment.
14. [ ] A SECOND or SUBSEQUENT preliminary amendment
15. [ ] A substitute specification.
16. [ ] A change of power of attorney and/or address letter.
17. [ ] A computer-readable form of the sequence listing in accordance with PCT Rule 13ter 2 and 35 U S.C. 1.821 - 1.825.
18. [X] A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. [ ] A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. [ ] Other items or information.

Express Mail EL 839703700 US

JC18 Rec'd PCT/PTO 12 FEB 2002
10/049393 .062902

U.S. APPLICATION NO (If known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/049393 To be assigned	PCT/EP00/07694	O-1999.475 US

	CALCULATIONS PTO USE ONLY	
21. [X] The following fees are submitted		
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)):		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO .. **$890.00**		
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	13 - 20 =	0	x **$18.00**	$
Independent claims	3 - 3 =	0	x **$84.00**	$
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ **$280.00**	$

TOTAL OF ABOVE CALCULATIONS =	$	
[ ] Applicant claims small entity status. See 37 CFR 1.27 The fees indicated above are reduced by 1/2. +	$	
SUBTOTAL =	$ 890.00	
Processing fee of **$130.00** for furnishing the English translation later than [ ] 20 [ ] 30 months from the earliest claimed priority date (37 CFR 1.492(f)).	$	
TOTAL NATIONAL FEE =	$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)) The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$	
TOTAL FEES ENCLOSED =	$ 890.00	
Amount to be refunded:	$	
charged:	$	

a. [ ] A check in the amount of $ _____ to cover the above fees is enclosed.

b. [X] Please charge my Deposit Account No. 02-2334 in the amount of $ 890.00 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. [X] The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No 02-2334. A duplicate copy of this sheet is enclosed

d. [ ] Fees are to be charged to a credit card **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO

Mark Milstead
Intervet Inc
Patent Department
P.O. Box 318
Millsboro, DE 19966

Mark W. Milstead
SIGNATURE

Mark W Milstead
NAME

45,825
REGISTRATION NUMBER

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:
Antonius BOM et al.

Serial Number: To be assigned Group: To be assigned

Filed: February 12, 2002 Examiner: To be assigned

For: USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK

Corresponding to: PCT/EP00/07694, filed August 7, 2000

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents
Washington, D.C. 20231

February 12, 2002

Sir:

Prior to the calculation of the fee in the above-identified application, please make the following amendments:

IN THE SPECIFICATION:

Please add the following subject heading on page 1, line 3:

--Field of the Invention--

Please add the following subject heading on page 1, line 7:

--Background of the Invention--

Please add the following subject heading on page 3, line 7:

--Brief Summary of the Invention--

Please add the following subject heading on page 3, line 21:

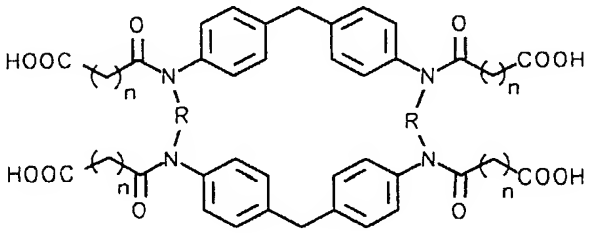
--Detailed Description of the Invention--

In the Claims

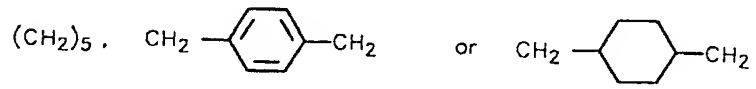
Please cancel claims 1-6 without prejudice or disclaimer of the subject matter contained therein.

Please amend the claims as follows:

7. (Amended) A cyclophane compound having formula A

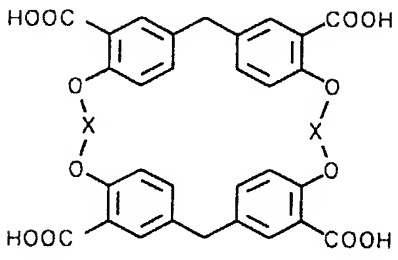


wherein R is

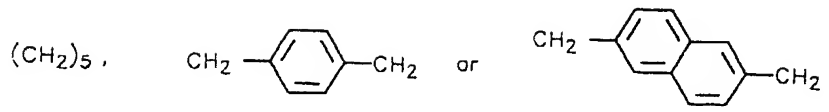


;

formula B



wherein X is



;

or a pharmaceutical acceptable salt thereof.

10. (Amended) The kit according to claim 9, wherein the neuromuscular blocking agent is rocuronium and the chemical chelator is γ-cyclodextrin or a derivative thereof.

11. (Amended) A method for reversal of drug-induced neuromuscular block in a patient, comprising:

parentally administering to said patent an effective amount of a chemical chelator capable of forming a guest-host complex with the drug inducing the neuromuscular block in a patient.

Please add the following claims:

--12. The method according to claim 11, wherein the drug inducing the neuromuscular block in the patient is rocuronium, vecuronium, pancuronium, rapacuronium, mivacuriam, (cis)atracuriam, tubocurarine or suxamethonium.

13. The method according to claim 11, wherein the chemical chelator is selected from the group consisting of cyclic oligosaccarides and cyclophanes.

14. The method according to claim 11, wherein the drug inducing neuromuscular block in a patient is rocuronium and the chemical chelator is γ-cyclodextrin or a derivative thereof.

15. A pharmaceutical composition, comprising:

a chemical chelator capable of reversing a drug-induced neuromuscular block, and

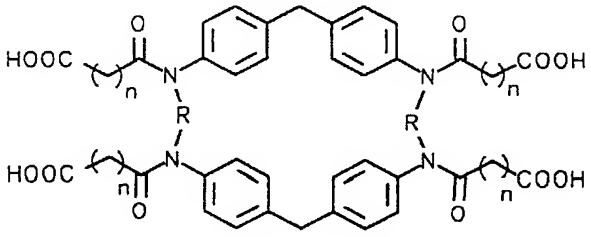
and a pharmaceutically acceptable excipient.

16. The pharmaceutical composition according to claim 15, wherein the chelator is selected from the group consisting of cyclic oligosaccharides and cyclophanes.

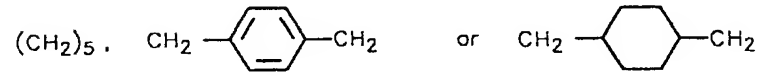
17. The pharmaceutical composition according to claim 16, wherein the cyclic oligosaccharide is a cyclodextrin or a derivative thereof.

18. The pharmaceutical composition according to claim 17, wherein the cyclodextrin is γ-cyclodextrin or a derivative thereof.

19. The pharmaceutical composition according to claim 16, wherein the cyclophane is represented by formula A

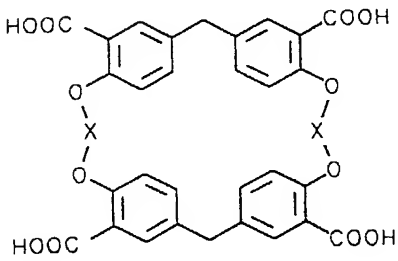


wherein R is

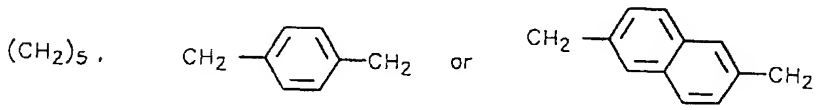


and n is 1-5; or

formula B



wherein X is



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Attorney Docket No. O/1999.475 US

REMARKS

Upon entry of the above preliminary amendment, claims 7-19 will be pending in the instant application. Applicants have canceled calms 1-6. Applicants have amended the specification by inserting subject heading in the proper locations. Claims 7, 10 and 11 have been amended to correct typographical errors and syntax. Original claims 9 and 10 provide support for new claims 12-14. Original claims 1-6 provide support for new claim 15-19. Applicants have not inserted any new matter.

Attorney Docket No. O/1999.475 US

Conclusion

It is believed that claims 7-19 recite a patentable improvement in the art. Favorable action is solicited.

Attached hereto is a marked-up version of the changes made to the application by this Preliminary Amendment.

In the event any fees are required with this paper, please charge our Deposit Account No. 02-2334, for which purpose duplicate copies are enclosed.

Respectfully submitted,

Mark W. Milstead
Registration No. 45,825

Intervet Inc.
Patent Department
405 State Street
P.O. Box 318
Millsboro, DE 19966
Tel: (302) 934-4395
Fax: (302) 933-4013

Attorney Docket No. O/1999.475 US
MWM

Enclosure: Version with Markings to Show Changes Made

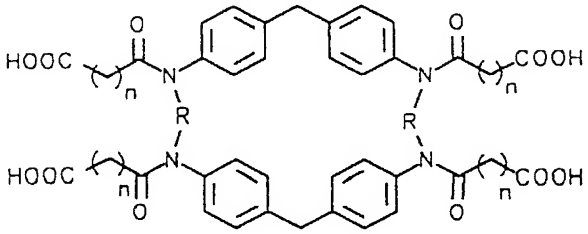
Attorney Docket No. O/1999.475 US

Version with Markings to Show Changes Made

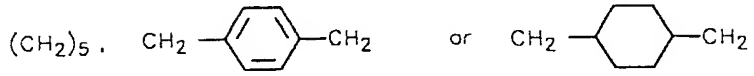
In the Claims

Claims 1-6 have been canceled.

7. (Amended) [The] A cyclophane [derivative] compound having [the general] formula A

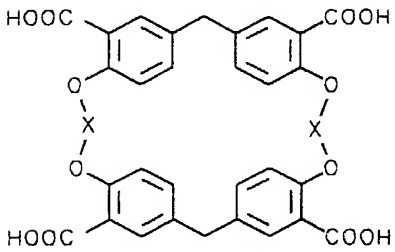


wherein R is

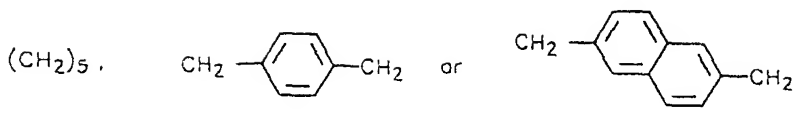


; [or]

[the general] formula B



wherein X is



[,];

or a pharmaceutical acceptable salt thereof.

10. (Amended) The kit according to claim 9, wherein the neuromuscular blocking [agenty] agent is rocuronium and the chemical chelator is γ-cyclodextrin or a derivative thereof.

11. (Amended) A method for reversal of drug-induced neuromuscular block in a patient, [which comprises] comprising:

parentally administering to said patent an effective amount of a chemical chelator capable of forming a guest-host complex with [said drug] the drug inducing the neuromuscular block in a patient.

Claims 12-19 have been added.

Use of Chemical Chelators as Reversal Agents for Drug-Induced Neuromuscular Block.

The invention relates to the use of chemical chelators for the preparation of a medicament for the reversal of drug-induced neuromuscular block, and to a kit for providing neuromuscular block and its reversal.

A neuromuscular blocking agent (NMBA, also called a *muscle relaxant*) is routinely used during the administration of anaesthesia to facilitate endotracheal intubation and to allow surgical access to body cavities, in particular the abdomen and thorax, without hindrance from voluntary or reflex muscle movement. NMBAs are also used in the care of critically-ill patients undergoing intensive therapy, to facilitate compliance with mechanical ventilation when sedation and analgesia alone have proved inadequate.

Based on their mechanisms of action, NMBAs are divided into two categories: depolarizing and non-depolarizing. Depolarizing neuromuscular blocking agents bind to nicotinic acetylcholine receptors (nAChRs) at the neuromuscular junction in a way similar to that of the endogenous neurotransmitter acetylcholine. They stimulate an initial opening of the ion channel, producing contractions known as fasciculations. However, since these drugs are broken down only relatively slowly by cholinesterase enzymes, compared to the very rapid hydrolysis of acetylcholine by acetylcholinesterases, they bind for a much longer period than acetylcholine, causing persistent depolarization of the end-plate and hence a neuromuscular block. Succinylcholine (suxamethonium) is the best known example of a depolarizing NMBA.

Non-depolarizing neuromuscular blocking agents compete with acetylcholine for binding to muscle nAChRs, but unlike depolarizing NMBAs, they do not activate the channel. They block the activation of the channel by acetylcholine and hence prevent cell membrane depolarization, and as a result, the muscle will become flaccid. Most clinically-used NMBAs belong to the non-

depolarizing category. These include tubocurarine, atracurium, (cis)atracurium, mivacurium, pancuronium, vecuronium, rocuronium and rapacuronium (Org 9487).

At the end of surgery or a period of intensive care, a reversal agent of NMBAs is often given to the patient to assist the recovery of muscle function. Most commonly used reversal agents are inhibitors of acetylcholinesterase (AChE), such as neostigmine, edrophonium and pyridostigmine. Because the mechanism of action of these drugs is to increase the level of acetylcholine at the neuromuscular junction by inhibiting the breakdown of acetylcholine, they are not suitable for reversal of depolarizing NMBAs such as succinylcholine. The use of AChE inhibitors as reversal agents leads to problems with selectivity, since neurotransmission to all synapses (both somatic and autonomic) involving the neurotransmitter acetylcholine is potentiated by these agents. This non-selectivity may lead to many side-effects due to the non-selective activation of muscarinic and nicotinic acetylcholine receptors, including bradycardia, hypotension, increased salivation, nausea, vomiting, abdominal cramps, diarrhoea and bronchoconstriction. Therefore in practice, these agents can be used only after or together with the administration of atropine (or glycopyrrolate) to antagonize the muscarinic effects of acetylcholine at the muscarinic receptors in the autonomic parasympathetic neuroeffector junctions (e.g. the heart).The use of a muscarinic acetylcholine receptor (mAChR) antagonist such as atropine causes a number of side-effects, e.g., tachycardia, dry mouth, blurred vision, and furthermore may affect cardiac conduction.

A further problem with anticholinesterase agents is that residual neuromuscular activity must be present (>10 % twitch activity) to allow the rapid recovery of neuromuscular function. Occasionally, either due to hypersensitivity of the patient or accidental overdose, administration of NMBAs can cause complete blockade of neuromuscular function ("deep block"). At present, there is no reliable treatment to reverse such a 'deep block'. Attempts to overcome a 'deep block' with high doses of AChE inhibitors has

the risk of inducing a "cholinergic crisis", resulting in a broad range of symptoms related to enhanced stimulation of nicotinic and muscarinic receptors.

There is thus a need for an alternative method for reversing the action of NMBAs, i.e. to restore the muscular contractions.

The present invention provides for the use of chemical chelators (or sequestrants) as reversal agents. In one aspect the invention pertains to the use of a chemical chelator capable of forming a guest-host complex for the manufacture of a medicament for the reversal of drug-induced neuromuscular block.

The use of chemical chelators as reversal agents for NMBAs has the advantage that they are effective in reversing the action of both depolarizing and non-depolarizing NMBAs, since chemical chelators do not compete with the NMBA for binding to nAChRs. Their use does not increase the level of acetylcholine and therefore they produce fewer side effects than AChE-based reversal agents. In addition, there is no need for the combined use of a AChE inhibitor and a mAChR antagonist (e.g., atropine). The chemical chelators of the invention may further be safely employed for the reversal of 'deep block'.

The term chemical chelator (or sequestrant), as used in the present invention, means any organic compound which can engage in host-guest complex formation with a neuromuscular blocking agent. The chemical chelator acts as the host molecule, the neuromuscular blocking agent being the guest molecule. The specific molecular complex, the guest-host complex, is defined as an organised chemical entity resulting from the association of two or more components held together by noncovalent intermolecular forces.

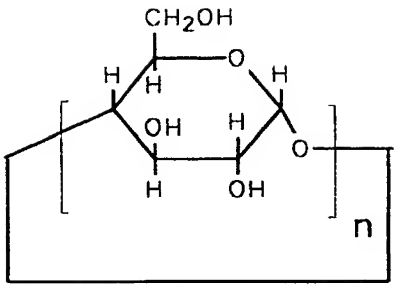
The chemical chelators (or sequestrants), according to the invention, are host molecules selected from various classes of, mostly cyclic, organic compounds which are known for their ability to form inclusion complexes with various

organic compounds in aqueous solution, e.g. cyclic oligosaccharides, cyclophanes, cyclic peptides, calixarenes, crown ethers and aza crown ethers. Formation of inclusion complexes (also called encapsulation, or chemical chelation) is part of the well-known area of 'supramolecular chemistry' or 'host-guest chemistry'. Many cyclic organic compounds are known to be capable of forming an inclusion complex with another molecule, organic or inorganic. The structures and chemistry of these compounds are well documented (*Comprehensive Supramolecular Chemistry*, Volumes 1-11, Atwood J.L., Davies J.E.D., MacNicol D.D., Vogtle F., eds; Elsevier Science Ltd., Oxford, UK, 1996).

Preferred chemical chelators for use with the present invention are cyclic oligosaccharides, cyclophanes and calixarenes.

Examples of cyclic oligosaccharides suitable for use with the invention are the cyclodextrins, a catagory of naturally occcurring cyclomaltooligosaccharides, the cyclomannins (5 or more α-D-mannopyranose units linked at the 1,4 positions by α linkages), the cyclogalactins (5 or more β-D-galactopyranose units linked at the 1,4 positions by β linkages), the cycloaltrins (5 or more α-D-altropyranose units linked at the 1,4 positions by α linkages), each of which are capable of forming guest-host complexes. Cyclic oligosaccharides of different monosaccharide compositions, accessible through total chemical synthesis, represent further chemical chelators capable of interaction with a neuromuscular blocking agent. For example, cyclo-[(1-4)-α-L-rhamno-pyranosyl-(1-4)-α-D-mannopyranosyl]tetraoside, was found to be effective in reversal of the action of the neuromuscular blocking agent rocuronium bromide.

A particularly preferred class of cyclic oligosaccharide chelators according to the invention is formed by the cyclodextrins:



n = 6 - 9

Cyclodextrins are cyclic molecules containing six or more α-D-glucopyranose units linked at the 1,4 positions by α linkages as in amylose. As a consequence of this cyclic arrangement, the cyclodextrins exist as conical shaped molecules with a lipophilic cavity which can attract guest molecules whilst the outside is more hydrophilic and water-soluble. Cyclodextrins composed of six, seven, eight and nine glucopyranose units are commonly known as α-, β-, γ- and δ-cyclodextrins, respectively.

Both the native cyclodextrins (α, β, γ) which are prepared by enzymatic degradation of starch, and especially a number of chemically modified forms thereof, have already found, by virtue of their ability to form guest-host complexes, numerous applications, especially in the pharmaceutical field. Stella and Rajewski (Pharmaceutical Research, 14, 556-567, 1997) have recently reviewed pharmaceutical applications of the cyclodextrins. The major applications are in the pharmaceutical formulations of drugs in order to solubilize and/or to stabilize a drug for oral, nasal, ophthalmic, dermal, rectal and parenteral administration.

The term cyclodextrin as used in relation to the present invention includes both the native cyclodextrins and chemically modified forms thereof.

An overview on such chemically modified cyclodextrins as drug carriers in drug delivery systems is described by Uekama et al. (Chemical Reviews 1998, 98, 2045-2076). Chemical modification of cyclodextrins can be made directly on the native α-, β- or γ-cyclodextrin rings by reacting a chemical reagent (nucleophiles or electrophiles) with a properly functionalised cyclo-

dextrin (for an recent overview of methods for the selective modification of cyclodextrins see Khan A.R. et al. *Chem. Rev.* 1998, 98, 1977-1996). To date, more than 1,500 cyclodextrin derivatives have been made by chemical modification of native cyclodextrins (Jicsinszky L. et al *Comprehensive Supramolecular Chemistry*, Volume 3. Cyclodextrins, Atwood J.L., Davies J.E.D, MacNicol D.D., Vogtle F., eds; Elsevier Science Ltd., Oxford, UK, 1996, pp 57-188).

Many direct modifications of a native cyclodextrin result in a mixture of isomers without precisely defined positions of substitution. Such a mixture of positional isomers is often referred to as a statistic mixture, the number of substituents attached at each cyclodextrin molecule in such a statistic mixture being expressed as the average degree of substitution (DS). Most cyclodextrin derivatives studied for pharmaceutical applications are statistic mixtures (Szente L. and Szejtli J., Adv. Drug Delivery Rev. 1999, 36, 17-28). Direct modification of a cyclodextrin does not alter the constitution or the configuration of the repeating α-D-glucopyranosyl units.

Cyclodextrins can also be prepared by *de novo* synthesis, starting with glucopyranose (Gattuso G. et al *Chem. Rev.* 1998, 98, 1919-1958). In this way, one can prepare not only the naturally occurring cyclic (1→4)-linked cyclodextrins but also the cyclic (1→3)-, (1→2)-, and (1→6)-linked oligopyranosides. Such a synthesis can be accomplished by using various chemical reagents or biological enzymes such as cyclodextrin transglycosylase. By using different sugar units as the starting materials, one can thereby prepare various homogeneous or heterogeneous cyclic oligosaccharides.
Chemical modification of cyclodextrins is thus known to modulate their properties and can be used for the design of reversal agents selective for a specific neuromuscular blocking agent.
It will be clear to the skilled person that for a particular neuromuscular blocking agent a chemical chelator host can be developed having a hydrophobic cavity of a shape and size adapted to the guest molecule, while

in addition to the hydrophobic interactions between the host and the guest charge interactions can be of importance for complex formation. Since the chemical chelators of the invention are for parenteral application they will have to be water-soluble. A specific host molecule can be designed and prepared to contain functionalities complementary to those of the guest molecule in such a manner that it results in maximum intermolecular interaction via for example hydrogen-bond, hydrophobic, electrostatic, van der Waals, and π-π interactions. Thus, for example, for a guest molecule containing basic functional groups or positive charge, a host molecule containing acidic functional groups or negative charge could be made to increase ionic interaction between the guest and the host. When such a host-guest complex is formed via inclusion or partial inclusion, the cavity size of the host molecule is also very important

The interaction between a chemical chelator and a neuromuscular blocking agent can be analyzed by physical methods such as nuclear magnetic resonance spectroscopy (nmr) and microcalorimetry.

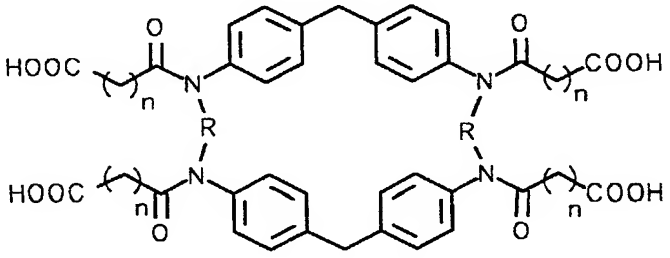
The most preferred cyclodextrins for use in the invention are γ-cyclodextrin and derivatives thereof.

Many of the commonly used neuromuscular blocking agents, or muscle relaxants, such as rocuronium, pancuronium, vecuronium, mivacurium, atracurium, (cis)atracurium, succinylcholine and tubocurarine, are compounds having 1 or 2 cationic sites when in neutral aqueous medium. Cyclodextrins having anionic sites in their structure are among the preferred chemical chelators according to the invention.

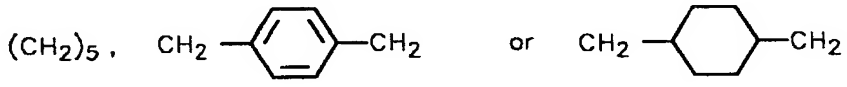
The preference for anionic chemical chelators for the reversal of the above mentioned neuromuscular blocking agents also applies for chemical chelators of the invention which belong to the cyclophanes.

Cyclophanes are a class of bridged aromatic compounds which have well-defined hydrophobic inclusion cavities constructed by aromatic rings incorporated in their macrocyclic structures. By introducing polar and hydrophilic functional groups such as hydroxyls and carboxyls into the artificial host compounds, cyclophanes can be made water-soluble and suitable for forming inclusion complex in aqueous media (Vogtle F. et al. *Comprehensive Supramolecular Chemistry*, Volume 2. Molecular recognition: Receptors for molecular guests, Atwood, J.L., Davies, J.E.D., MacNicol, D.D., Vogtle, F., eds; Elsevier Science Ltd., Oxford, UK, 1996, pp 211-266). Water soluble anionic cyclophanes are described by Miyake et al. (Tetr. Letters 32, 7295-7298, 1991; Chem. Pharm. Bull. 41, 1211-1213, 1993) as hosts for cationic aromatic guests. Analogously, cationic cyclophanes were shown to form inclusion complexes in aqueous solution with anionic and neutral aromatic compounds.

In a preferred embodiment of the invention the chemical chelator is chosen from cyclophanes having the general formula A

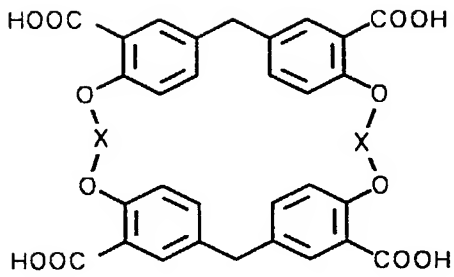


wherein R is

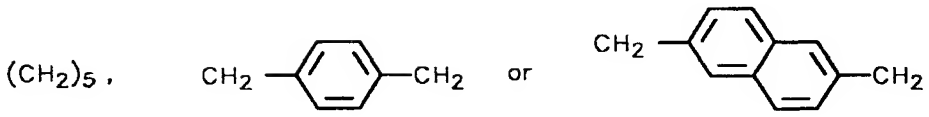


and n is 1-5; or the general formula B

9



wherein X is



The compound according to formula A, wherein R is CH_2-phenyl-CH_2 and n is 2, i.e. N, N', N'', N'''-tetrakis (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,-29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (compound **23**), and the compound according to formula B wherein X is CH_2-phenyl-CH_2, i.e. 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy-[2.2.1.2.2.1]-paracyclophane (compound **18**) is a preferred cyclophane derivative to reverse the action of rocuronium bromide.

In a further aspect the invention provides for novel paracyclophane derivatives having formula A or Formula B, as defined above, or pharmaceutically acceptable salts thereof. Examples of such salts are the potassium, sodium and ammonium salts and the like.

The chemical chelators for use in the invention are administered parenterally. The injection route can be intravenous, subcutaneous, intradermal, intramuscular, or intra-arterial. The intravenous route is the preferred one. The exact dose to be used will neccessarily be dependent upon the needs of the individual subject to whom the medicament is being administered, the degree of muscular activity to be restored and the judgement of the anaesthetist/critical-care specialist. Extracorporal application of the chemical

chelators of the invention, for instance by mixing of the chemical chelator with the blood during dialysis or during plasmapheresis, is also contemplated.

In a further aspect the invention relates to a kit for providing neuromuscular block and its reversal comprising (*a*) a neuromuscular blocking agent, and (*b*) a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent. With a kit according to the invention is meant a formulation, which contains separate pharmaceutical preparations, i.e. the neuromuscular blocking agent and a chemical chelator, i.e. the reversal agent. The components of such a kit of parts are to be used sequentially, i.e. the neuromuscular blocking agent is administered to a subject in need thereof, which is followed, at a point in time when restoration of muscle function is required, by the administration of the reversal agent, i.e. a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent.

A preferred kit, according to the invention, contains a chemical chelator selected from the group consisting of a cyclic oligosaccharide and a cyclophane, and a neuromuscular blocking agent which is selected from the group consisting of rocuronium, vecuronium, pancuronium, rapacuronium, mivacurium, atracurium, (cis)atracurium, tubocurarine and suxamethonium.
A particularly preferred kit of the invention comprises rocuronium, as the neuromuscular blocking agent, and γ-cyclodextrin, or a derivative thereof, as the chemical chelator.

Mixed with pharmaceutically suitable auxiliaries and pharmaceutically suitable liquids, e.g. as described in the standard reference, Gennaro et al., Remington's Pharmaceutical Sciences, (18th ed., Mack Publishing Company, 1990, Part 8: Pharmaceutical Preparations and Their Manufacture; see especially Chapter 84 on "Parenteral preparations, pp. 1545-1569; and Chapter 85 on "Intravenous admixtures", pp. 1570-1580) the chemical

chelators can be applied in the form of a solution, e.g. for use as an injection preparation.

Alternatively, the pharmaceutical composition may be presented in unit-dose or multi-dose containers, for example sealed vials and ampoules, and may be stored in a freeze dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example, water prior to use.

The invention further includes a pharmaceutical formulation, as hereinbefore described, in combination with packaging material suitable for said composition, said packaging material including instructions for the use of the composition for the use as hereinbefore described.

The invention is illustrated in the following examples.

Example 1.

Cyclodextrin derivatives.

Some of the cyclodextrin derivatives that were employed to demonstrate their activity as reversal agents according to the invention were commercially available:

	Commercial Source
Compound **1**: α-cyclodextrin (α-CD)	Wacker-Chemie GmbH, Munich, Germany; or ALDRICH
Compound **2**: carboxymethyl-β-CD (DS= 3.5) sodium salt	Wacker-Chemie GmbH, Munich, Germany
Compound **3**: 2-hydroxy-3-trimethylammonio propyl--β-CD (DS= 3.5)	Wacker-Chemie GmbH, Munich, Germany
Compound **4**: per 2,6-dimethyl--β-CD (DS=12.6)	Wacker-Chemie GmbH, Munich, Germany
Compound **5**: β-cyclodextrin-phosphate sodium salt (DS=3)	CycloLab, Ltd. Budapest, Hungary
Compound **6**: β-cyclodextrin-phosphate sodium salt (DS=8)	CycloLab, Ltd. Budapest, Hungary
Compound **7**: carboxymethyl-β-CD (DS= 3-3.5)	CycloLab, Ltd. Budapest, Hungary
Compound **8**: carboxyethyl-β-CD (DS= 3)	CycloLab, Ltd. Budapest, Hungary
Compound **9**: β-cyclodextrin (β-CD)	Wacker-Chemie GmbH, Munich, Germany; or ALDRICH
Compound **10**: 2-hydroxypropyl-β-CD	RBI, Natick, MA 01760, USA
Compound **11**: γ-cyclodextrin-phospate sodium salt (DS=3)	CycloLab, Ltd. Budapest, Hungary
Compound **12**: γ-cyclodextrin-phospate sodium salt (DS=7)	CycloLab, Ltd. Budapest, Hungary
Compound **13**: carboxymethyl-γ-CD (DS=3.2)	CycloLab, Ltd. Budapest, Hungary
Compound **14**: carboxyethyl-γ-CD (DS= 3.8)	CycloLab, Ltd. Budapest, Hungary
Compound **15**: γ-cyclodextrin (γ-CD)	Wacker-Chemie GmbH, Munich, Germany; or FLUKA
Compound **16**: 2-hydroxypropyl-γ-CD (DS= 4)	RBI, Natick, MA 01760, USA

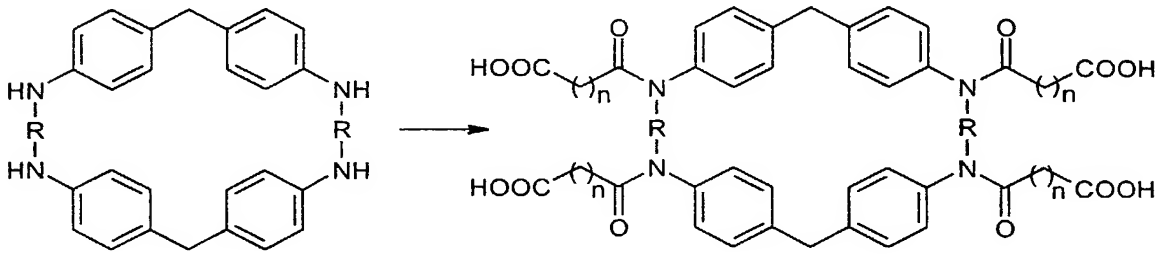
- DS means the degree of substitution, which is the mean number of hydroxy functions which carry the pertinent substituent.
- compounds **2** and **7** are the same, be it from different suppliers.

Example 2.

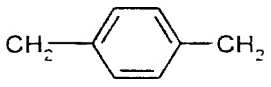
Cyclophane derivatives.

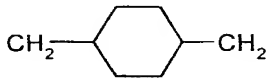
Nomenclature: the term *para*cyclophane refers to a family of compounds in which one or more benzene rings are built into a carbocyclic ring system and in which the *p*-positions of the benzene rings are part of the ring system. The conventional numbering used below for *para*cyclophane ring systems is that described by Cram and Abell (J.Am.Chem.Soc. 1955, 77, 1179-1186) __

A: Tetraaza-paracyclophanes derivatives:



I: R = $(CH_2)_5$

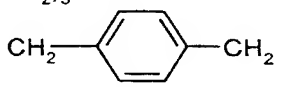
II: R = 

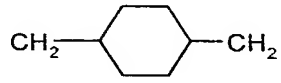
III: R = 

Compound 19: R = $(CH_2)_5$, n = 1

Compound 20: R = $(CH_2)_5$, n = 3

Compound 21: R = $(CH_2)_5$, n = 2

Compound 22: R =  n = 2

Compound 23: R =  n = 2

Scheme A

Scheme A depicts the structures of the N-(carboxy)acylated cyclophane derivatives **19**-**23**, which were prepared by acylation of the parent cyclophanes (see Soga T. et al *Tetrahedron Lett.* 1980, 4351-4, for synthesis thereof) (1,7,21,27-tetraaza[7.1.7.1]paracyclophane (**I**), 1,10,24,33-tetraaza-[2.2.1.2.2.1]paracyclophane (**II**) and 3,4,5,6,7,8,26,27,28,29,30,31-dodeca-

hydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (**III**) with the appropriate activated acid derivative.

A1: Compound **21**:

N, N', N'', N'''-Tetrakis(3-carboxypropionyl)-1, 7, 21, 27-tetraaza[7.1.7.1]para - cyclophane.

To a suspension of 1,7,21,27-tetraaza[7.1.7.1]paracyclophane (400 mg, 0.75 mmol) in dichloromethane (5 ml) was added triethylamine (1.05 ml, 7.52 mmol) followed by 3-(methoxycarbonyl)propionyl chloride (0.93 ml, 7.52 mmol) dissolved in dichloromethane (3 ml). The reaction was stirred under an atmosphere of nitrogen for 12 h. The reaction was diluted with dichloromethane (20 ml) and washed with water (2 x 20 ml), dried ($MgSO_4$) and the solvent removed *in vacuo* to give a yellow oil, which was purified by chromatography on silica gel eluting with 5% methanol in dichloromethane. The resultant product crystallised on standing. The product was recystallised from chloroform/ether to give N, N', N'', N'''-tetrakis[3-(methoxycarbonyl)-propionyl]-1, 7, 21, 27-tetraaza[7.1.7.1]para cyclophane (480 mg, 0.48 mmol, 65%). MS (EI) m/z 989 $(M+H)^+$, ^1H NMR ($CDCl_3$) δ 1.27 (m, 4H), 1.45 (m, 5H), 1.64 (m, 3H), 2.25 (t, J 6.8, 8H), 2.56 (t, J 6.8, 8H), 3.61 (t, J 7.6, 8H), 3.65 (s, 12H), 4.02 (s, 4H), 7.09 (d, J 8.1. 8H), 7.20 (d, J 8.1, 8H); ^{13}C NMR ($CDCl_3$) δ 24.45, 27.85, 29.17, 29.37, 40.73, 49.47, 51.68, 128.47, 130.27, 140.15, 140.60, 171.35, 173.84.

A mixture of the above tetramethylester (440 mg, 0.45 mmol), potassium hydroxide pellets (2.51 g, 45 mmol), methanol (9 ml) and water (25 ml) was heated to reflux for 4 h. The reaction was cooled to room temperature, most of the solvent removed *in vacuo* and the residue acidified with 2N HCl. The resultant precipitate was filtered and dried then recystallised from $MeOH/H_2O$ to give the title compound **21** (142 mg, 0.15 mmol, 34%).

MS (EI) m/z 931 $(M-H)^-$, ^1H NMR (CD_3OD) δ 1.28 (m, 4H), 1.44 (m, 8H), 2.25 (m, 8H), 2.49 (m, 8H), 3.60 (m, 8H), 4.05 (s, 4H), 7.16 (d, J 7.72, 8H), 7.30 (d, J 7.72, 8H), ^{13}C NMR ($CDCl_3$) δ 23.95, 27.44, 28.94, 29.11, 39.86, 48.62, 128.21, 129.79, 140.01, 140.35, 170.01, 173.07, I.R. (KBr) 1736, 1656 cm^{-1}.

In a similar manner were prepared:

A2: N, N', N'', N'''-Tetrakis(carboxyacetyl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane (**Compound 19**) starting from 1, 7, 21, 27-tetraaza[7.1.7.1]-paracyclophane and methyl malonyl chloride.

MS (EI) m/z 877 $(M+H)^+$, ^1H NMR (DMSO) δ 1.21 (m, 4H), 1.36 (m, 8H), 2.97 (m, 8H), 3.53 (m, 8H), 3.99 (s, 4H), 7.15 (d, J 7.95, 8H), 7.27 (d, J 7.95, 8H), 12.40 (s, 4H), ^{13}C NMR (DMSO) δ 23.79, 27.19, 39.39, 41.44, 48.59, 128.04, 129.83, 145.05, 141.02, 165.69, 169.23, I.R. (KBr) 1736, 1625 cm^{-1}.

A3: N, N', N'', N'''-Tetrakis(4-carboxybutyryl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane (**Compound 20**) starting from 1,7,21,27-tetraaza[7.1.7.1]-paracyclophane and methyl 4-(chloroformyl)butyrate.

MS (EI) m/z 989 $(M+H)^+$, ^1H NMR (CD_3OD) δ 1.28 (m, 4H), 1.44 (m, 8H), 1.76 (m, 8H), 2.07 (t, J 7.5, 8H), 2.19 (t, J 7.5, 8H), 3.62 (m, 8H), 4.05 (s, 4H), 7.11 (d, J 8.17, 8H), 7.27 (d, J 8.17, 8H), ^{13}C NMR ($CDCl_3$) δ 21.86, 25.54, 28.72, 34.02, 34.41, 41.70, 50.60, 129.50, 131.47, 141.82, 142.55, 174.69, 177.69, I.R. (KBr) 1736, 1656 cm^{-1}.

A4: N, N', N'', N'''-Tetrakis(3-carboxypropionyl)1,10,24,33-tetraaza-[2.2.1.2.2.1]paracyclophane (**Compound 22**) starting from cyclophane **II** and 3-(methoxycarbonyl)propionyl chloride.

^1H NMR (DMSO) δ 2.22 (m, 8H), 2.42 (m, 8H), 3.93 (s, 4H), 4.75 (s, 8H), 6.96 (d, J 8.27, 8H), 7.00 (s, 8H), 7.16 (d, J 8.27, 8H), 11.90 (bs, 1H), ^{13}C NMR (DMSO) δ 28.83, 29.10, 39.93, 51.51, 125.32, 127.83, 128.22, 129.43, 136.36, 139.88, 14.00, 170.69, 173.52, I.R. (KBr) 1727, 1650 cm^{-1}.

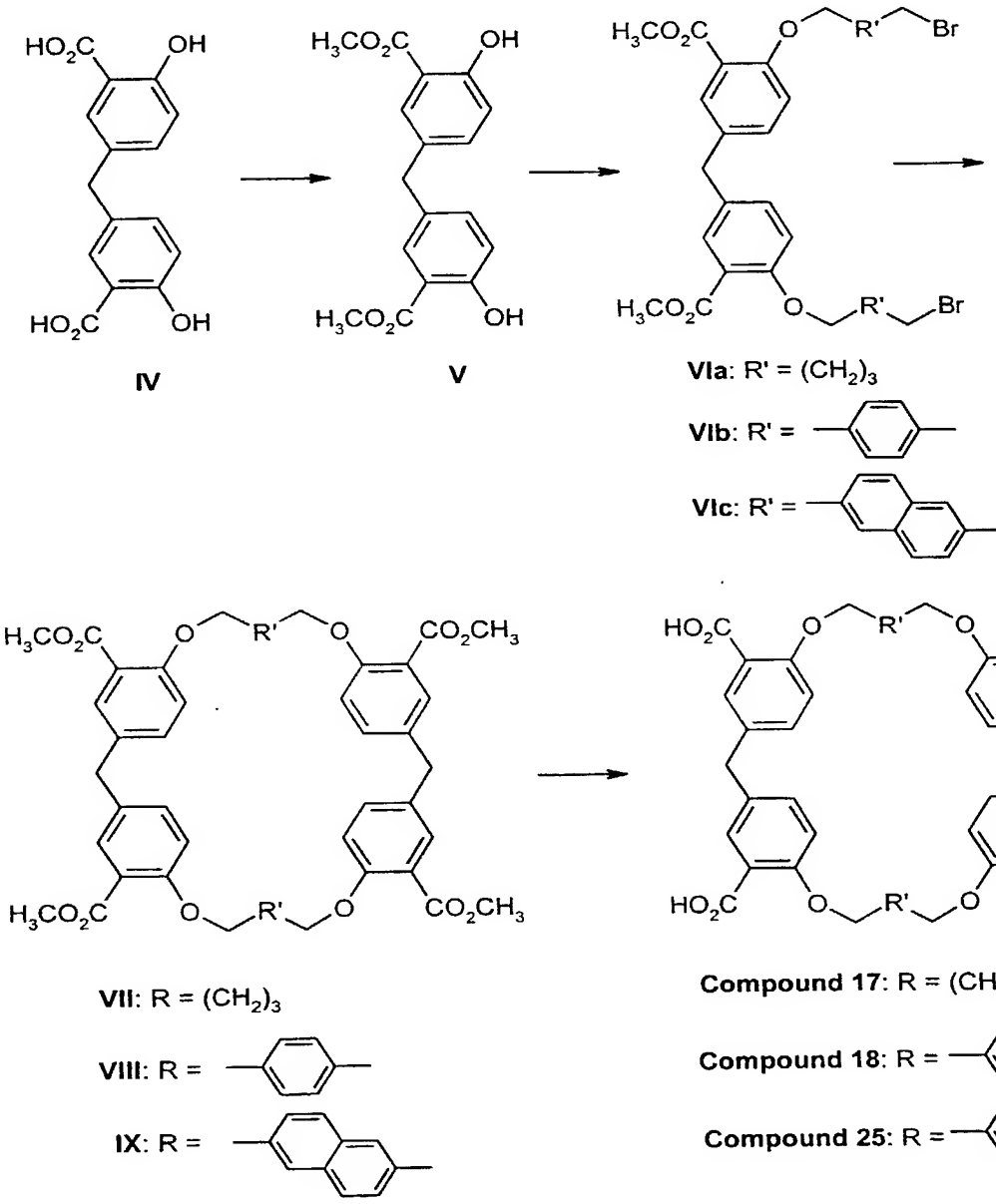
A5: N, N', N'', N'''-Tetra (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane (**Compound 23**) starting from cyclophane **III** and 3-(methoxycarbonyl)propionyl chloride.

MS (EI) m/z 1012 $(M-H)^-$, ^1H NMR (DMSO) δ 0.76 (m, 8H), 1.51 (m, 4H), 1.67 (m, 8H), 2.15 (m, 8H), 2.36 (t, J 6.60, 8H), 3.37 (s, 8H), 3.97 (s, 4H), 7.19 (d,

J 8.08, 8H), 7.30 (d, J 8.08, 8H), 11.86 (bs, 4H), ^{13}C NMR (DMSO) δ 28.97, 29.09, 36.13, 52.33, 55.59, 128.09, 129.72, 141.55, 170.65, 173.52, I.R. (KBr) 1727, 1652 cm^{-1}

B. Tetraoxa-paracyclophane derivatives.

The ether-linked cyclophanes (compounds **17**, **18** and **25**) can be synthesised by a ring construction as shown in **Scheme B**.



Scheme B

The commercially available 5,5'-methylenedisalicylic acid **IV** was protected as methyl ester **V** which was then alkylated with an appropriate dihalide to yield **VI**. Reaction of dihalide **VI** with an equivalent diphenol **IV** gave the cyclophanes **VII-IX** which gave the desired carboxylic acid derivatives upon saponification.

B1: Compound **17**: 1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy[7.1.7.1]para-cyclophane.

3,3'–Dimethoxycarbonyl-4, 4'-dihydroxydiphenylmethane (**V**)

To methanol (100 ml) saturated with hydrogen chloride gas was added 3,3'–dicarboxy-4,4'-dihydroxydiphenylmethane (10 g, 34.69 mmol) portion-wise over 30 min. The mixture was then heated to reflux for 3 h, cooled to room temperature and re-saturated with hydrogen chloride gas. After a further 8 h heating at reflux the solvent was removed *in vacuo* and the product purified by chromatography on silica gel eluting with 25% ethyl acetate/petroleum ether to give the title compound (2.40 g, 7.59 mmol, 22%). ^{1}H NMR ($CDCl_3$) δ 3.84 (s, 2H), 3.92 (s, 6H), 6.90 (d, J 8.0, 2H), 7.25 (dd, J 8.0, 1.0, 2H), 7.63 (d, J 1.0, 2H), 10.65 (s, 2H).

4,4'-Bis(5-bromopentoxy)-3,3'–dicarboxymethyl-4,4'-dihydroxydiphenyl-methane (**VIa**)

To a stirred suspension of 1, 4-dibromopentane (21.8 g, 94.9 mmol) and K_2CO_3 (13.1 g, 94.9 mmol) in dry dimethylformamide (380 ml) at 60 °C, under an atmosphere of nitrogen, was added dropwise a solution of 3,3'–dicarboxymethyl-4,4'-dihydroxydiphenylmethane (3.0 g, 9.49 mmol) in dry dimethylformamide(190 ml). The resultant mixture was heated a further 1h, cooled and filtered. The dimethylformamide was removed *in vacuo* and the product purified by chromatography eluting with 1% methanol in dichloro-methane followed by a second purification eluting with 10% ethyl acetate/-heptane to give the title compound (3.51 g, 5.73 mmol, 60%).

^{1}H NMR ($CDCl_3$) δ 1.60-2.02 (m, 12H), 3.44 (t, J 7.0, 4H), 3.87 (s, 8H), 4.01 (t, J 6.5, 4H), 6.87 (d, J 8.0, 2H), 7.22 (dd, J 8.0, 1.0, 2H), 7.60 (d, J 1.0, 2H).

1,7,21,27-tetraoxa-9,17,29,37-tetra(methoxycarbonyl)[7.1.7.1]paracyclophane (**VII**)

A solution of 4,4'-bis(5-bromopentoxy)-3,3'–dicarboxymethyl-4,4'-dihydroxydiphenylmethane (3.51 g, 5.74 mmol) and 3,3'–dicarboxymethyl-4, 4'-dihydroxydiphenylmethane (1.81 g, 5.74 mmol) in dry dimethylformamide (230 ml) was added dropwise *via* syringe pump to a stirred suspension of K_2CO_3 (7.92 g, 57.4 mmol) in dry dimethylformamide (340 ml) at 80 °C over a period of 3 h. After stirring a further 4.5 h at 80 °C and 12 h at room temperature the reaction mixture was filtered and the dimethylformamide removed *in vacuo*. The product was purified by chromatography on silica gel eluting with 1% methanol/dichloromethane to give the title compound (0.47 g, 0.6 mmol, 10.5%).

NMR ($CDCl_3$) δ 1.58-1.95 (m, 12H), 3.82 (s, 16H), 4.05 (t, J 8.0, 8H), 6.82 (d, J 8.0, 4H), 7.16 (dd, J 8.0, 1.0, 4H), 7.58 (d, J 1.0, 4H).

1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy[7.1.7.1]paracyclophane (**17**)

To a suspension of 1,7,21,27-tetraoxa-9,17,29,37-tetra(methoxycarbonyl)[7.1.7.1]paracyclophane (0.47 g, 0.612 mmol) in methanol-water (3:1, 40 ml) was added solid sodium hydroxide (0.49 g (12.2 mmol). The resultant mixture was heated to reflux for 1h, then tetrehydrofuran (5 ml) was added and the mixture again heated to reflux for 2 h. The solvent volume was reduced by half *in vacuo* and insoluble material removed by filtration. The filtrate was acidified with conc hydrochloric acid and the resultant precipitate filtered dried, and further washed with methanol-water before drying to give the title compound (160 mg, 0.22 mmol, 45%).

MS (EI) m/z 711 $(M-H)^-$, ^{1}H NMR (DMSO) δ 1.51 (m, 4H), 1.68 (m, 8H), 3.81 (s, 4H), 3.99 (t, J 5.6, 8H), 6.96 (m, 4H), 7.20 (m, 4H), 7.43 (m, 4H), 12.25 (bs, 4H), ^{13}C NMR (DMSO) δ 21.57, 27.81, 38.72, 68.47, 113.99, 121.77, 130.28, 132.60, 133.21, 155.61, 167.27, I.R. (KBr) 1743 cm^{-1}

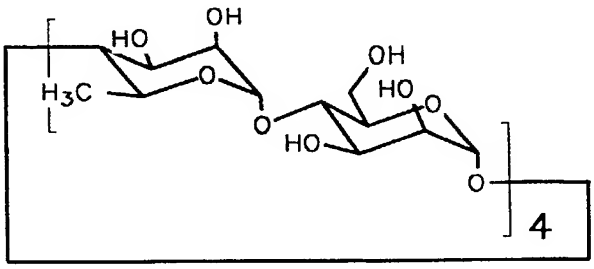
In a similar manner were prepared

B2: 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy-[2.2.1.2.2.1]paracyclophane, compound **18**, starting from 3,3'–dicarboxymethyl-4,4'-dihydroxydiphenyl methane and α,α'-dibromo-*p*-xylene.
MS (EI) m/z 779 $(M-H)^-$, ^1H NMR (DMSO) δ 3.75 (s, 4H), 5.15 (s, 8H), 6.91 (d, J 8.58, 4H), 7.20 (m, 4H), 7.32 (m, 8H), 7.49 (m, 4H), 12.40 (bs, 4H), ^{13}C NMR (DMSO) δ 68.69, 84.57, 114.52, 126.54, 129.98, 132.32, 133.81, 136.28, 154.60, 167.50, I.R. (KBr) 1733 cm^{-1}

B3: Compound **25** starting from 3,3'–dicarboxymethyl-4,4'-dihydroxydiphenyl methane and 2, 6-(dibromomethyl)naphthalene (Golden, J. H., J. Chem. Soc. 1961, 3741).
MS (EI) m/z 879 $(M-H)^-$, ^1H NMR (DMSO) ? 3.76 (s, 4H), 5.30 (s, 8H), 6.90 (m, 4H), 7.19 (m, 4H), 7.49 (m, 8H), 7.74 (m, 4H), 7.83 (m, 4H), 12.60 (bs, 4H), ^{13}C NMR (DMSO) ??69.21, 83.84, 114.73, 121.88, 125.25, 125.37, 127.96, 130.34, 132.04, 132.67, 133.97, 135.03, 154.91, 167.52, I.R. (KBr) 1731 cm^{-1}.

Example 3.

Compound **24**: cyclo[(1-4)-α-L-rhamnopyranosyl-(1-4)-α-D-mannopyranosyl]-tetraoside.



The synthesis of this cyclic octasaccharide is described by Ashton et al. in Chem. Eur. J. 1996, 2, 580-591.

Example 4

Complexation of Rocuronium bromide by chemical chelators.

All the ^1H spectra (303 K) were recorded at 400.13 MHz with 128 scans, sw = 12 ppm, TD = 32 k and zero filled to 64 k real points in processing. All experiments were measured at 303K.

Determination of stoichiometry

Stock solutions of rocuronium bromide and γ-cyclodextrin (**15**) were prepared, both with a concentration of 6.02 mM. From these, sixteen solutions were prepared (with the mole % of rocuronium ranging from 0-100) by taking aliquots of 0-800 µl of the rocuronium bromide solution. Aliquots of the γ-cyclodextrin solution, 800-0 µl were added to make the solution a total volume of 800 µl and 100 mole % (i.e. 6.02 mM of [rocuronium bromide + γ-cyclodextrin]). ^1H-NMR spectra were recorded as described above.

If the chemical shift change of $H_{9\alpha}$ in rocuronium bromide is defined as Δδ then a plot of [Δδ*(mole % rocuronium bromide)] vs. [mole % rocuronium bromide] gives a so called Job Plot by the method of continuous variation (Connors K.A.: *Binding constants, The measurement of Molecular Complex Stability*; Wiley-Interscience; New York, 1987, pp 24-28). The maximum in this plot is indicative for the stoichiometry of the complex. The Job Plot for the rocuronium bromide/γ-cyclodextrin complex has a maximum at 50 mole % rocuronium, indicating that rocuronium bromide and γ-cyclodextrin form a 1:1 complex.

Determination of the association constants.

A stock solution of rocuronium bromide: 0.821 mM in D_2O was prepared. Stock solutions of β-cyclodextrin (**9**) and γ-cyclodextrin (**15**), both with concentrations of 13.1, 6.57, 1.64, and 0.411 mM in D_2O, were prepared. Aliquots of 50-400 µl of these solutions were then removed and made up to 400 µl with D_2O (where required) and mixed with 400 µl of the rocuronium bromide solution. To extend the data range for γ-cyclodextrin (**15**)

experiments to higher cyclodextrin concentrations three additional solutions were prepared: 16.4, 24.6, 32.8 mM in 400 µl D_2O. As before, these solutions were mixed with 400 µl of the rocuronium bromide solution.

^1H NMR spectra were recorded as descibed above.

The association constants of the complexes were derived from the plots of proton chemical shift changes of the cyclodextrin and/or rocuronium bromide signals ($\Delta\delta$) versus the mole % cyclodextrin, using a curve fitting method (Loukas Y.L., J. Pharm. Pharmacol. 1997, 49, 941; Bisson A.P., et al. Chem. Eur. J. 1998, 4, 845). The association constants are listed in Table are listed in Table A.

Table A. Association constants of 1:1 complex of rocuronium bromide and cyclic host compounds (K_a, M^{-1}), determined by NMR spectroscopy at 303 K.

Chemical chelator	*Association constant (K_a, M^{-1})*
β-cyclodextrin (**9**)	3,100 – 3,900*
γ-cyclodextrin (**15**)	10,000 – 20,400#

*protons of CH_3-19 [rocuronium] and H_3 [β-cyclodextrin (**9**)] measured.

#protons of $H_{9\alpha}$ [rocuronium] and $H_{3,5}$ [γ-cyclodextrin (15) measured.

Example 5.

Reversal of neuromuscular blockade *in vivo*:

Anaesthetized guinea pig.

Male Dunkin-Hartley guinea pigs (bodyweight: 600-900 g) were anaesthetized by i.p. administration of 10 mg/kg pentobarbitone and 1000 mg/kg urethane. After tracheotomy, the animals were artificially ventilated using a Harvard small animal ventilator. A catheter was placed into the carotid artery for

continuous monitoring of arterial blood pressure and the taking of blood samples for blood gas analysis. Heart rate was derived from the blood pressure signal. The sciatic nerve was stimulated (rectangular pulses of 0.5 ms duration at 10 s (0.1 Hz) intervals at a supramaximal voltage, using a Grass S88 Stimulator) and the force of M. gastrocnemius contractions was measured using a Grass FT03 force-displacement transducer. Contractions, blood pressure and heart rate were recorded on a multichannel Grass 7D recorder. Catheters were placed in both jugular veins. One catheter was used for the continuous infusion of a neuromuscular blocking agent. The infusion rate of the neuromuscular blocking agent was increased until a steady-state block of 85-90% was obtained. The other catheter was used for administration of increasing doses of the reversal agent. During continuous infusion of the neuromuscular blocking agent, single doses of increasing concentration of reversal agent were given. At the end of the experiment, the measured force of muscle contractions was plotted against the concentration of reversal agent, and using regression analysis techniques, the 50% reversal concentration was calculated.

Results for the reversal of the neuromuscular block, induced by the muscle relaxants rocuronium bromide (Roc), vecuronium bromide (Vec), pancuronium bromide (Pan), mivacurium chloride (Miv), atracurium besilate (Atr), cis-atracurium (Cis-Atr), tubocurarine chloride (T-C), suxamethonium chloride (Sux; succinylcholine) and rapacuronium bromide (Rap; Org 9487), by means of a series of α-, β- and γ-cyclodextrins (compounds **1** - **16**) and modified cyclodextrins are presented in **Table I**. The results demonstrate that the action of each of the neuromuscular blocking agents can be reversed by intravenous administration of a cyclodextrin derivative.

TABLE I Dose (ED_{50}, $\mu mol.kg^{-1}$) producing 50% reversal of steady-state neuromuscular block in anaesthetized guinea pigs.

Compound*	Roc	Vec	Pan	Miv	Atr	Cis-Atr	T-C	Sux	Rap
1: α-cyclodextrin (α-CD)	1575 (2)	2610 (1)							
2: carboxymethyl-β-CD (DS= 3.5) sodium salt	134 (2)	800 (1)							
3: 2-hydroxy-3-trimethylammonio propyl--β-CD (DS= 3.5)	518 (2)	1400 (1)							
4: per 2,6-dimethyl--β-CD (DS=12.6)	70 (2)	1313 (4)							
5: β-cyclodextrin-phosphate sodium salt (DS=3)	280 (3)								
6: β-cyclodextrin-phosphate sodium salt (DS=8)	120 (2)								
7: carboxymethyl-β-CD (DS= 3-3.5)	139 (3)								
8: carboxyethyl-β-CD (DS= 3)	42 (3)	103 (2)	479 (2)					408 (2)	412 (2)
9: β-cyclodextrin (β-CD)	20 (3)	636 (1)	1050 (2)						358 (3)
10: 2-hydroxypropyl-β-CD	33 (3)	1598 (4)							
11: γ-cyclodextrin-phospate sodium salt (DS=3)	64 (3)	67 (3)	197 (2)	292 (2)	113 (2)	204 (2)	160 (2)	160 (2)	80 (3)
12: γ-cyclodextrin-phospate sodium salt (DS=7)	52 (2)								
13: carboxymethyl-γ-CD (DS=3.2)	25 (4)	30 (3)	641 (4)	964 (3)	990 (2)	893 (2)	431 (2)	999 (2)	227 (2)
14: carboxyethyl-γ-CD (DS= 3.8)	7 (3)	25 (3)	141 (3)	399 (2)	421 (2)	1558 (3)	294 (2)	1294 (2)	43 (3)
15: γ-cyclodextrin (γ-CD)	4 (3)	75 (4)	186 (4)	1690 (2)	2793 (4)	1710 (2)	630 (2)	1189 (2)	57 (3)
16: 2-hydroxypropyl-γ-CD (DS= 4)	12 (3)	123 (3)	440 (3)	1674 (2)	828 (2)	878 (2)	909 (2)	1340 (2)	134 (3)

ED_{50} values are the mean of a number of experiments; the number is shown in parenthesis.

Example 6.

Reversal of neuromuscular block *in vitro* :

Isolated mouse hemidiaphragm preparation.

Hemidiaphragms, with phrenic nerves attached, were removed from euthanized male mice (Institute of Cancer Research; bodyweight: 20-60 g). The preparations were mounted on a tissue holder and placed in a tissue bath filled with a modified Krebs-Henseleit solution (composition: 118 mM NaCl, 30 mM NaHCO3, 5 mM KCl, 1 mM KH_2PO_4, 1 mM $MgSO_4$, 30 mM glucose and 2.5 mM $CaCl_2$) at 37°C and bubbled with 95% oxygen and 5% carbondioxide. One end of the preparation was connected with a siliconized silk suture to a Grass FT03 force-displacement transducer. An initial force of 10 mN was applied. The phrenic nerve was placed on a bipolar platinum electrode and was stimulated with rectangular pulses of 0.2 ms duration at 20 s (0.05 Hz) intervals at a supramaximal voltage, using a Grass S88 Stimulator. Contractions were recorded on a four channel Grass 79D recorder.

After the development of stable contractions, an appropriate single dose of a neuromuscular blocking agent was added to each bath to produce inhibition of contractions to approximately 5-10% of baseline values after 20 min contact time (this concentration was found to be 3.1 μM for rocuronium bromide). Increasing amounts of reversal agent were then administered into the bath at intervals of 10 min. The % maximum reversal was established. At the end of the experiment, the measured muscle contractions force was plotted against the concentration of reversal agent, and using regression analysis techniques, the 50% reversal concentration was calculated.

After induction of neuromuscular block by rocuronium, the % maximum reversal produced by the addition of a number of γ-cyclodextrin derivatives (compounds **11**-**16**), a cyclic octasaccharide comprising 4 rhamnosyl-manno-pyranosyl units (compound **24**), or a number of paracyclophane derivatives (compounds **17**-**21** and **23**) are presented in Table II. The results demonstrate that the neuromuscular blocking action of rocuronium can be efficiently

blocked by chemical chelators of variable structure, i.e. by the γ-cyclodextrins **13**, **14** and **15**, by a cyclic oligosaccharide composed of rhamnose and mannose (**24**), and by action of the cyclophanes **18** and **23**.

TABLE II Mouse hemidiaphragm: maximum reversal (%)

Compound	3.1 μM rocuronium[1]
CYCLODEXTRINS	
11: γ-cyclodextrin-phospate sodium salt (DS=3)	34 (360)
12: γ-cyclodextrin-phospate sodium salt (DS=7)	17 (360)
13: carboxymethyl-γ-CD (DS=3.2)	88 (180)
14: carboxyethyl-γ-CD (DS= 3.8)	87 (144)
15: γ-cyclodextrin (γ-CD)	94 (144)
16: 2-hydroxypropyl-γ-CD (DS= 4)	71 (360)
CYCLIC OLIGOSACCHARIDE	
24: cyclo[(1-4)-α-L-rhamnopyranosyl-(1-4)-α-D-mannopyranosyl]tetraoside	86 (288)
CYCLOPHANES	
17: 1,7,21,27-tetraoxa-9,17,29,37-tetracarboxy-[7.1.7.1]paracyclophane	11 (360)
18: 1,10,24,33-tetraoxa-12,20,35,43-tetracarboxy--[2.2.1.2.2.1]paracyclophane	61 (288)
19: N, N', N", N"'-tetrakis(carboxyacetyl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane	2.4 (108)
20: N, N', N", N"'-tetrakis(4-carboxybutyryl)-1, 7, 21, 27-tetraaza[7.1.7.1] paracyclophane	8.7 (72)
21: N, N', N", N"'-tetrakis(3-carboxypropionyl)-1, 7, 21, 27-tetraaza[7.1.7.1]para cyclophane	0 (360)
23: N, N', N", N"'-tetrakis (3-carboxypropionyl)-3,4,5,6,7,8,26,27,28,29,30,31-dodecahydro-1,10,24,33-tetraaza[2.2.1.2.2.1]paracyclophane	90 (58)

[1]: concentration of chemical chelator at maximum reversal in μM in parenthesis;

Example 7.

Calixarene derivatives.

4-Sulfonic calix[6]arene and 4-sulfonic calix[8]arene were obtained from Aldrich.

Reversal of neuromuscular block *in vivo*, induced by rocuronium bromide, was carried out as described in Example 5. The dose (ED_{50}) of the calixarene derivative producing 50 % reversal of steady-state neuromuscular block in the anaesthetised guinea pig was found to be 5.1 μmol/kg for the 4-sulfonic calix[6]arene and 34 μmol/kg for the 4-sulfonic calix[8]arene.

Reversal of neuromuscular block *in vitro* was carried out using the mouse hemidiaphragm preparation as described in Example 6. After induction of neuromuscular block (95 % block) with rocuronium bromide (3.6 μM in the bath) maximum reversal of 124 % and 120 % were recorded for 4-sulfonic calix[8]arene and the 4-sulfonic calix[6]arene , respectively, while the 50% reversal concentrations were found to be 36 μM and 34 μM.

Claims.

1. Use of a chemical chelator for the manufacture of a medicament for the reversal of drug-induced neuromuscular block.

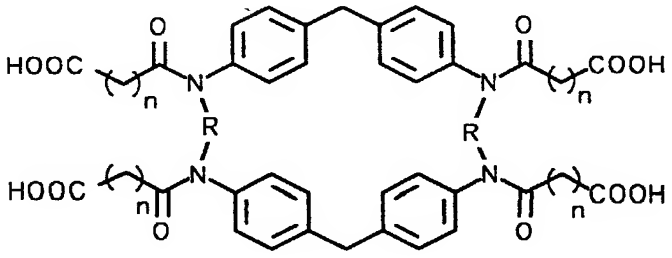
2. The use according to claim 1, wherein the chelator is selected from the group consisting of cyclic oligosaccharides, cyclophanes and calixarenes.

3. The use according to claim 2, wherein the chelator is a cyclic oligosaccharide.

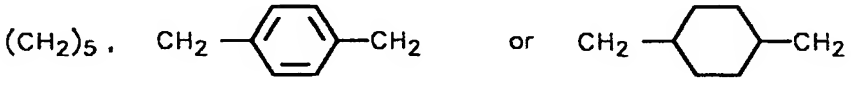
4. The use according to claim 3, wherein the cyclic oligosaccharide is a cyclodextrin or a derivative thereof.

5. The use according to claim 4, wherein the cyclodextrin is γ-cyclodextrin or a derivative thereof.

6. The use according to claim 2, wherein the cyclophane has the general formula A

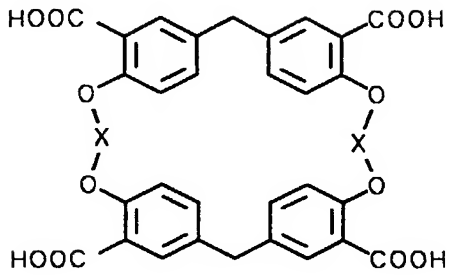


wherein R is

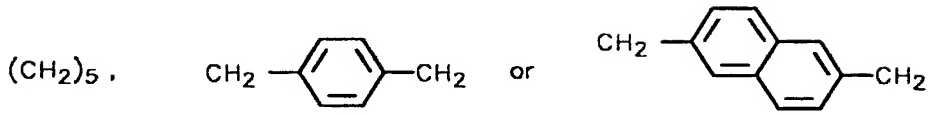


and n is 1-5; or

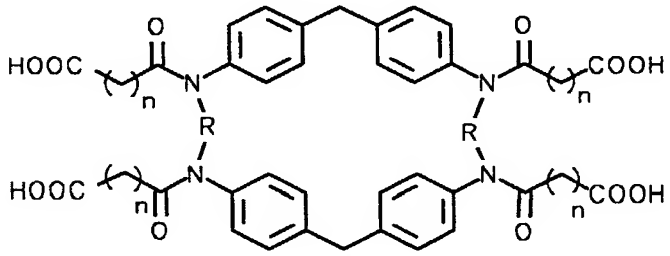
the general formula B



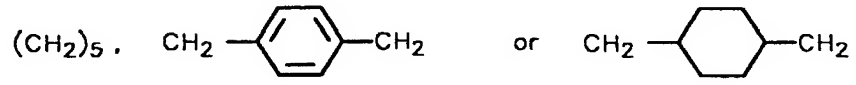
wherein X is



7. The cyclophane derivative having the general formula A

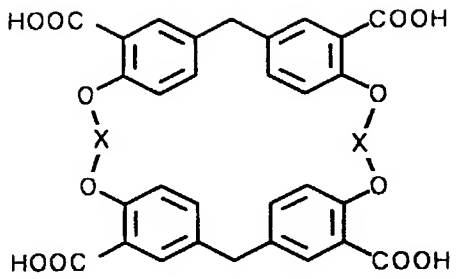


wherein R is

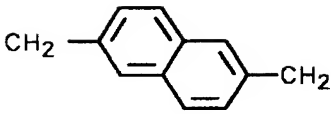


; or

the general formula B



wherein X is

$(CH_2)_5$,  or 

or a pharmaceutically acceptable salt thereof.

8. A kit for providing neuromuscular block and its reversal comprising (*a*) a neuromuscular blocking agent, and (*b*) a chemical chelator capable of forming a guest-host complex with the neuromuscular blocking agent.

9. The kit according to claim 8, wherein the neuromuscular blocking agent is selected from the group consisting of rocuronium, vecuronium, pancuronium, rapacuronium, mivacurium, (cis)atracurium, tubocurarine and suxamethonium, and wherein the chelator is selected from the group consisting of cyclic oligosaccharides, cyclophanes and calixarenes.

10. The kit according to claim 9, wherein the neuromuscular blocking agenty is rocuronium and the chemical chelator is γ-cyclodextrin or a derivative thereof.

11. A method for reversal of drug-induced neuromuscular block in a patient which comprises parenterally administering to said patient an effective amount of a chemical chelator capable of forming a guest-host complex with said drug.

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(71) Applicant *(for all designated States except US)*: **AKZO NOBEL N.V.** [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BOM, Antonius, Helena, Adolf** [NL/GB]; 16 Ratho Park Road, Ratho, Midlothian EH28 8NY (GB). **MUIR, Alan, William** [GB/GB]; Howfaulds Farm, Ravenstruther, Lanark ML11 7SL (GB). **REES, David** [GB/SE]; Jacobsdalsgatan 40, S-412 68 Göthenburg (SE).

(74) Agent: **KRAAK, H.**; P.O. Box 20, NL-5340 BH Oss (NL).

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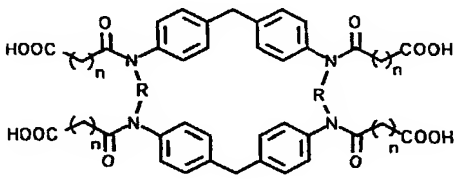

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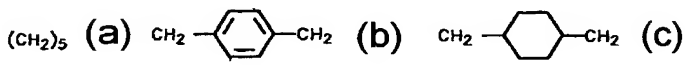

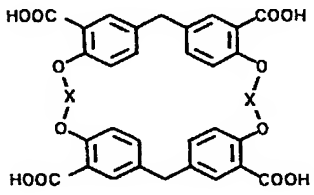

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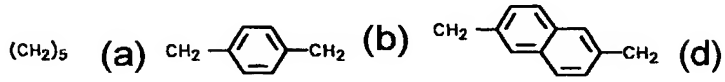

(57) Abstract: The invention relates to the use of chemical chelators for the preparation of a medicament for the reversal of drug-induced neuromuscular block, to a kit for providing neuromuscular block and its reversal, and to cyclophane derivatives having general formula (A) wherein R is (a), (b) or (c); or general formula (B) wherein X is (a), (b) or (d), or a pharmaceutically acceptable salt thereof.

WO 01/12202 A2

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter for which a patent is sought on the invention entitled:

"USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK"

the specification of which
[CHECK ONE]
[ ]is attached hereto

[ ] was filed on_____as Application Serial No.

_____and was amended on_____
[if applicable]

[X]as filed under the Patent Cooperation Treaty on **07 August 2000**

Serial **PCT/EP/00/07694** The United States of America being designated.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

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Prior Foreign Application(s)			Priority	claimed
99306411.2	**EP**	**13/August/1999**	**[X]Yes**	**[ ]No**
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Number	Country	Day/Month/Year filed		
		/ /	**[ ]Yes**	**[ ]No**
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And I hereby appoint as principal attorney, William M. Blackstone, Registration No. 29,772 William Peterson Ramey III, Registration No. 44,295 and Mark W. Milstead, Registration No. 45,825

Please address all communications to:

William M. Blackstone
INTERVET INC.
405 State Street
MILLSBORO, DE 19966
USA

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Full name of sole or first inventor **BOM, Antonius, Helena, Adolf**

Inventor' signature_____

Citizenship **DUTCH** **Date**

Residence and P.O.Address **16 Ratho Park Road**
Ratho, Midlothian
EH28 8 NY Scotland
United Kingdom

Full name of sole or first inventor **MUIR, Alan, William**

Inventor's signature _____

Date

Citizenship **BRITISH**

Residence and P.O.Address **Howfaulds Farm**
Ravenstruther, Lanark
ML11 7SL,Scotland
United Kingdom

Full name of third joint inventor **REES, David**

Inventor's signature _____ AUGUST 13 2002

Date

Citizenship **BRITISH**

Residence and P.O.Address **Cedergatan 32 A**
S-426 74 Göthenburg
Sweden

Full name of forth joint inventor ___

Inventor's signature _____

Date

Citizenship _____

Resid Residence and P.O.Address

Full name of fifth joint inventor

Inventor's signature _____

Date

Citizenship _____

Resid Residence and P.O.Address

Full name of sixth joint inventor _____

Inventor's signature _____

Date

Citizenship _____

Residence and P.O.Address _____

Full name of seventh joint inventor _____

Inventor's signature _____

Date

Citizenship _____

Residence and P.O.Address _____

Full name of eighth joint inventor _____

Inventor's signature _____

Date

Citizenship _____

Residence and P.O.Address _____

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"USE OF CHEMICAL CHELATORS AS REVERSAL AGENTS FOR DRUG-INDUCED NEUROMUSCULAR BLOCK"

the specification of which
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[ ]is attached hereto

[ ] was filed on _____ as Application Serial No.

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I acknowledge the duty to disclose to the Patent and Trademark Office all information known to me to be material to patentability as defined Title 37, Code of Federal Regulations Section 1.56(a)

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign applications(s) for patent or inventor's certificate having a filing date before that of the application(s) on which priority is claimed:

Prior Foreign Application(s)			Priority	claimed
99306411.2	**EP**	**13/August/1999**	**[X]Yes**	**[ ]No**
Number	Country	Day/Month/Year filed		
		/ /	**[ ]Yes**	**[ ]No**
Number	Country	Day/Month/Year filed		
		/ /	**[ ]Yes**	**[ ]No**
Number	Country	Day/Month/Year filed		

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application(s) in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose to the patent and Trademark

Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application.

(U.S. Serial No.)	(Filing date)	(Status-patented, pending, abandoned)
(U.S. Serial No.	(Filing date)	(Status-patented, pending, abandoned)

3 And I hereby appoint as principal attorney, William M. Blackstone, Registration No. 29,772 William Peterson Ramey III, Registration No. 44,295 and Mark W. Milstead, Registration No. 45,825

Please address all communications to:

William M. Blackstone
INTERVET INC.
405 State Street
MILLSBORO, DE 19966
USA

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued theron.

1-00

Full name of sole or first inventor **BOM, Antonius, Helena, Adolf**

Inventor' signature _____

Citizenship **DUTCH** Date 31/7/02

Residence and P.O.Address **16 Ratho Park Road**
Ratho, Midlothian
EH28 8 NY Scotland
United Kingdom GBN

2-00

Full name of sole or first inventor **MUIR, Alan, William**

Inventor's signature _____

Date 31/7/02

Citizenship **BRITISH**

Residence and P.O.Address **Howfaulds Farm**
Ravenstruther, Lanark
ML11 7SL,Scotland
United Kingdom GBN

3-00

Full name of third joint inventor **REES, David**

Inventor's signature

Date

Citizenship **BRITISH**

Residence and P.O.Address **Cedergatan 32 A**
S-426 74 Göthenburg
Sweden SE

Full name of forth joint inventor ___

Inventor's signature _____

Date

Citizenship _____

Resid Residence and P.O.Address

Full name of fifth joint inventor

Inventor's signature _____

Date

Citizenship _____

Resid Residence and P.O.Address

Full name of sixth joint inventor _____

Inventor's signature

Date

Citizenship _____

Residence and P.O.Address _____

Full name of seventh joint inventor _____

Inventor's signature

Date

Citizenship _____

Residence and P.O.Address _____

Full name of eighth joint inventor _____

Inventor's signature

Date

Citizenship _____

Residence and P.O.Address _____